

YALE UNIVERSITY LIBRARY

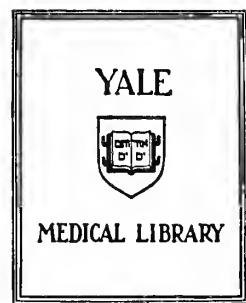


39002010050905

PENETRATION OF SPINALLY ADMINISTERED OPIOIDS
INTO THE LUMBAR SPINAL CORD OF CATS:
AN AUTORADIOGRAPHIC STUDY.

Joe Wayne Kurosu

1986



Permission for photocopying or microfilming of "Penetration
of Spinally Administered Opioids into the Lumbar Spinal
(title of thesis)
Cord of Cats: An Autoradiographic Study."

for the purpose of individual scholarly consultation or reference is hereby granted by the author. This permission is not to be interpreted as affecting publication of this work, or otherwise placing it in the public domain, and the author reserves all rights of ownership guaranteed under common law protection of unpublished manuscripts.

Joe W. Kurosu
(Signature of author)

Joe W. Kurosu
(Printed name)

March 7, 1986
(Date)



Digitized by the Internet Archive
in 2017 with funding from
Arcadia Fund

<https://archive.org/details/penetrationofspi00kuro>

PENETRATION OF SPINALLY ADMINISTERED
OPIOIDS INTO THE LUMBAR SPINAL CORD OF CATS:
AN AUTORADIOGRAPHIC STUDY.

A Thesis Submitted to the Yale University
School of Medicine in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Medicine

by

Joe Wayne Kurosu

1986

1986

Med Lib

713

712

5446

TABLE OF CONTENTS

	<u>PAGE</u>
Abstract.....	ii
Acknowledgements.....	iv
Introduction.....	1
History of Opium and its Medical Uses.....	4
The Opiate Receptor.....	8
Distribution of the Opiate Receptor.....	12
The Endogenous Opioids.....	16
Receptor Subtypes.....	20
Subtypes Within the Spinal Cord.....	20
Sites of Opioid Action.....	22
Spinal vs. Supraspinal Action of Opioids.....	22
Relative Significance of Spinal and Supraspinal Effects.....	27
Sites of Opioid Action Within the Spinal Cord.....	29
Clinical Use of Spinally Administered Opioids.....	32
Current Study: Autoradiographic Technique.....	35
Current Study: Aims.....	37
Methods.....	41
Results.....	47
Discussion.....	49
Figures & Tables.....	56
References.....	65

ABSTRACT

PENETRATION OF SPINALLY ADMINISTERED
OPIOIDS INTO THE LUMBAR SPINAL CORD OF CATS:
AN AUTORADIOGRAPHIC STUDY.

Joe Wayne Kurosu

1986

Although the analgesic action of opioids has, in the past, been attributed to supraspinal sites of action, evidence has now accumulated indicating direct actions at the spinal cord level. Animal and human studies have shown that spinally administered opioids can produce a powerful segmental analgesia without loss of other sensory or motor functions. The exact mechanisms by which spinally administered opioids give rise to this analgesia, however, are not entirely clear. This study was undertaken to develop an autoradiographic technique suitable for investigating the penetration of spinally administered morphine into the lumbar spinal cord of cats, and, by comparing the temporal changes in radio-labelled morphine localization with known electrophysiological effects of spinal morphine, gain a clearer understanding of the mechanisms behind spinal opioid analgesia. Following lumbar laminectomy on six cats of either sex, the dura mater was reflected, exposing the spinal cord. The cerebrospinal fluid was then suctioned off and replaced with a solution of ^3H -morphine (25 μCi in 25 μl) and unlabelled morphine sulfate (0.25 mg in 0.5 cc) in a total volume of 525 μl . After 30 seconds, 5 minutes, or 10 minutes, the lumbar spinal cord was rapidly removed, cut into 5 segments each about 2 mm thick and numbered 1-5 from rostral to caudal then immediately frozen in isopentane cooled to -65°C with dry ice. Sections (8 microns thick) of the frozen tissue were obtained in a cryostat at -15°C , placed on gelatin coated slides, and freeze-dried at -15°C under vacuum in a dessicator. Slides were then dipped into a liquid emulsion diluted 1:2 with double distilled deionized water and exposed for four weeks in light-tight boxes at -15°C . The autoradiographs were then developed, stained with 2.5% cresyl violet, and examined under a light microscope. Autoradiographic grain density per $625 \mu^2$ was obtained in selected areas (posterior column, laminae I, II, V, VII, and ventral horn) by visual counting. The technique used produced autoradiographs of good histological quality with uniformly low levels of radiographic background. Analysis of data by rostral-caudal segments did not reveal any consistent trends in grain distribution.

Data pooled without regard to segment number was thus analyzed, and showed that grains were present in amounts greater than background within 30 seconds after morphine application in all areas sampled. Furthermore, there was a significant increase ($P < .01$ by t-test for unpaired data) in grain density in all areas sampled between the 5-minute and 10-minute time intervals. The variability in grain counts, however, was felt to be relatively large. Thus, it was concluded that the autoradiographic technique used was satisfactory for the study of penetration of opioid compounds into the spinal cord, although the somewhat large variability in grain counts suggested that some movement of radioactive morphine was occurring during the tissue processing. Also, morphine, despite its low lipophilicity, was shown to penetrate to the deeper laminae of the spinal cord within minutes after application, but the study did not allow any conclusions to be drawn on the specific binding or action of the drug at these laminae. Finally, the temporal changes in autoradiographic grain density were concluded to be consistent with the known time course of suppression of dorsal horn WDR neurons by spinally applied morphine.

ACKNOWLEDGEMENTS

First and foremost, I would like to acknowledge the efforts of Professor Luke M. Kitahata for allowing me to utilize his resources and for his time, advice, patience and understanding in all aspects of this thesis project. In addition, I would like to thank Dr. Raymond S. Sinatra and Dr. Jerry G. Collins for their kind assistance, advice, and direction throughout all stages of this study. I am most indebted to my mentors in the laboratory, Drs. M. Aoki, Y. Kumeta, K. Murata, and Y. Nishio for their unlimited patience in helping me with the often time consuming "drudgery" of daily lab work. No part of this project would have been possible without their assistance. Finally, I would like to dedicate this thesis to my parents, Dr. & Mrs. Yoshio Kurosu, for whom words cannot adequately express my sense of respect, appreciation, and love.

"Now Helen, the daughter of Zeus, turned her thoughts elsewhere. Straightway, she cast into the wine of which they drank, a drug which quenches pain and strife and brings forgetfulness to every ill."

From The Odyssey of Homer.

INTRODUCTION

Opium has been known to man for at least 6000 years (59). Although it is likely to have been used initially for its psychological effects, specific medical applications were eventually developed (21), and many of the current uses of opioids were established by three or four hundred years ago (25). Insights into the mechanisms by which opium exerted its various effects, however, came somewhat more slowly. For example, morphine, the principle alkaloid contained in opium, was not isolated until the early 19th century (59), and it was not until much more recently that specific receptors for opioids were shown to exist in animal tissue (25). Furthermore, although it has been suspected for some time that opium and its derivatives acted predominantly on the nervous system (59), more precise localization of the sites of action of opioids has come only within the past ten to twenty years. Earlier studies concentrated on supraspinal areas as potential sites of action of opioids, but with the demonstration of opiate receptors in the spinal cord, discovery of the endogenous opioids, and evidence that opioids have direct effects on the spinal cord (38), it became apparent that opioids may exert their effects both at the spinal and supraspinal

levels. As Yaksh has pointed out (76), this discovery of the spinal action of opioids proved to be of "significant clinical importance," and clinical use and investigations of intrathecal or epidural opioids has become increasingly common (9). Many questions, however, still remain. One of these concerns the exact site of action of spinally administered opioids. There already exists some evidence pointing to specific areas of the spinal cord as sites where opioids may be exerting their effects (76). The present study aims to provide further evidence by investigating the time course of penetration of spinally administered morphine into the spinal cord and correlating these results with the known electrophysiological responses of spinal cord neurons to morphine applied spinally.

Before detailed discussion of the present study, it is felt that a review of some background material would be appropriate. In the introduction which follows, a brief history of opioids and their medical uses will be given, followed by a discussion of the concept of opiate receptors, theories on sites of opioid action, and, finally, a discussion of the proposed sites of action of spinally administered opioids. A brief discussion of the technique of autoradiography will also

be made. Research related to opioids has mushroomed in the past 10 to 15 years. The following discussion is thus in no way meant to be a comprehensive one but will serve as a background for the present study.

HISTORY OF OPIUM AND ITS MEDICAL USES

Crude opium is the white sap which exudes when the unripe seed capsules of the poppy plant (*Papaver somniferum*) is scratched. The term "opium" is the diminutive of the Greek "opos" which means sap (59). Opium itself contains over twenty distinct alkaloids including morphine, codeine, thebaine, papaverine, and noscapine (25). Curiously enough, this white sap is present in the plant for only ten days, after which it is broken down (59). Perhaps even more intriguing is why the plant produces this substance in the first place. The reason for this is not clear. We do know however, that opium "has affected the history of the human race, causing wars, threatening society, and bringing comfort to those in pain (59)."

As mentioned above, opium has been known to man for quite some time. The poppy is referred to as a "plant of joy" in Sumerian ideograms dating back to 4000 B.C. (21), and seeds of the cultivated poppy have been found in relics of Stone Age lake dwellers in Switzerland (59). The first known reference to opium as a medicine can be found in the Therapeutic Papyrus of Thebes from Egypt in 1552 B.C., which describes a mixture of fly excretions and opium prescribed for quieting crying babies (21). From Egypt, the poppy

found its way to Greece. Hippocrates (460-357 B.C.) seems to have been wary of the effects of opium, and although his Egyptian counterparts were prescribing the use of opium freely by this time, he was against the liberal use of such drugs (21). Dioscorides, another Greek physician, used opium not only as a soporific and analgesic, but also to treat such conditions as chronic cough (59).

The Romans used opium in increasing amounts, perhaps in part due to Galen (130-201 A.D.) who felt that it cured, among other things, "deafness, epilepsy, asthma, jaundice, fevers, melancholy, and all pestilences (59)." The Arabs are generally credited with spreading the use of opium both eastward and westward. Thus it was through them that during the sixth century opium was introduced into India, and from there into China. The image of opium in China is one of opium smoking addicts, but it was not until the 17th century when tobacco was prohibited that this mode of taking opium became popular in that country. Prior to this, opium was used as a medicine by only a select class of Chinese (59).

Meanwhile in Europe, opium continued to enjoy

great popularity. Paracelsus, a Swiss doctor of the early 16th century thought to be an opium addict himself, played an important role in popularizing the use of opium in Europe. He claimed that he possessed "a secret remedy...[called] laudanum...which is superior to all heroic remedies (25)." Franciscus Sylvius (1614-1672) after whom the "sylvian fissure" is named, once reported that he would not want to practice medicine without opium (21). Thomas Sydenham of England went so far as to say that "without opium the healing arts would cease to exist (59)."

As widespread use of opium continued, the search was beginning for the "basic principle" or "quintessence" of opium in hopes of procuring a more powerful form of the drug. Various extracts and preparations were concocted in this search without much success until the early 19th century. In 1803 Derosne, a French chemist, isolated a crystallizable salt from opium which he found to be more powerful than any other extract and called it Sel narcotique de Derosne (21). This salt was one of the alkaloids of opium, now known as narcotine or noscapine. Around the same time, a German named Friedrich Serturner had isolated an "element" from opium--a substance several times more powerful than crude opium--and named it morphium after Morpheus,

the Greek God of Sleep (25). Thus morphine had been isolated, and the isolation of other alkaloids from opium soon followed. As a result, use of the pure alkaloid forms rather than opium became popular in the medical world (25).

The next significant development came in 1853 when the hypodermic needle was perfected by Alexander Wood. This, of course, allowed for parenteral use of drugs, and morphine was no exception as its medical use by hypodermic injection was pioneered in the U.S. by Fordyce Barker and George T. Elliot (59).

Since that time, a variety of natural, synthetic, and semi-synthetic opioid drugs have come into use. In addition to their parenteral and oral use as analgesics, they have come to be employed in moderate doses as adjuncts to general anesthesia, in high doses as anesthetics for cardiac surgery, and most recently via the subarachnoid or epidural route for pain relief.

THE OPIATE RECEPTOR

Despite such a long relationship between man and opiates, detailed knowledge of the sites and modes of action of opiates has only begun to emerge recently. The principle site of action of opiates has long been thought to reside in the central nervous system, but further localization proved to be difficult until a major advance toward understanding of opiate action came in the early 1970's with the identification of the opiate receptor. The pharmacological action of most drugs assumes "that the initial process in drug action is the formation of a reversible complex between the drug and a cell component generally known as the drug receptor (5)." The highly selective effects which opiates produce at low concentrations suggests that this is also true for the opiates. Three lines of evidence lend further support to the concept of an opiate receptor (61):

1. Structure. All opiates have basic similarities in their molecular structure. Namely, the presence of a positively charged tertiary nitrogen, a flat phenolic base, and a hydroxyl group.
2. Stereospecificity. Although most opiates exist as at least two optical isomers, only the levorotatory forms give rise to the selective effects of opiates.
3. Antagonists. Minor molecular modifications of opiate agonists can give rise to antagonists, which block the actions of agonists without giving rise to any effects themselves.

Thus, investigators felt quite sure that specific opiate receptors existed, but initial studies aimed at localizing the receptor met with a major obstacle--the fact that nonspecific binding greatly exceeded specific receptor binding. That is, opiates, like many other compounds, bind in a nonspecific fashion to nearly any biological membrane, and do so to such an extent that the small portion which is specifically bound is difficult to detect.

Goldstein and his colleagues were the first to attempt to distinguish between specific and nonspecific opiate binding (22). This was accomplished by using the criteria of stereospecificity. Homogenized brain tissue from mice was incubated with ^3H -levorphanol in the presence of a pharmacologically active isomer (levorphanol) and an inactive isomer (dextrorphan). Figure 1 diagrams the conditions used by Goldstein and shows the three types of binding which he described: nonsaturable (trapped and dissolved), nonspecific saturable, and specific saturable. Trapped and dissolved opiates (C in Fig.1) accounted for nearly 50% of the total binding while nonspecific saturable binding (A minus B in Fig.1) was responsible for close to another 50%, leaving only 2% of total binding as that due to stereospecific binding (B minus C in Fig.1).

This result was somewhat discouraging for it indicated that it would be a difficult task indeed to isolate specific binding to the putative receptor in the presence of large amounts of nonspecific binding. Thus, methods were sought which would somehow "amplify" the specific binding relative to the nonspecific binding, making it easier to detect.

Pert and Snyder (54) were among the first to overcome this difficulty and demonstrate the existence of an opiate receptor in nervous tissue. They used low concentrations of an opiate antagonist with high specific activity (5×10^{-9} M of 3 H-naloxone) and washed the tissues rapidly with cold buffer after incubation. The use of small amounts of a high affinity drug favored specific binding, and the rapid washing helped to remove nonspecifically bound molecules. Samples were also incubated in the presence of dextrorphan and in the presence of levorphanol to "subtract" out any nonspecific binding which remained after the washing, similar to Goldstein's technique above. Using this method, they demonstrated highly stereospecific binding of low concentrations of opiates, with the degree of binding paralleling the known pharmacological potencies of opiate agonists and antagonists--strong evidence for the presence of

an opiate receptor.

Terenius (65) independently demonstrated high-affinity stereospecific binding in synaptic plasma membrane fractions of rat cerebral cortex, using a technique similar to Pert and Snyder's above, with ³H-dihydromorphine. Simon et al. (60) provided further evidence for the existence of opiate receptors in rat brain homogenate using, again, a similar technique and the potent agonist etorphine.

It should be noted that stereospecific binding, while highly suggestive, does not by itself constitute proof for the existence of a receptor (80). Stereospecific binding of opiates has, in fact, been demonstrated to cerebrosides (46) and even certain glass filters (62). Thus it was essential to demonstrate a correlation between the binding affinity and pharmacological activity for various opiates. Such a correlation was shown by Pert and Snyder (see above) and, perhaps more convincingly, by Creese and Snyder (13) who demonstrated an association between binding and pharmacological activity within the same system--the guinea-pig ileum. These investigators compared the ability of various opiates to inhibit electrically induced muscular contractions with their ability to inhibit naloxone binding,

both in the guinea-pig ileum. They were able to demonstrate a strong correlation between binding and pharmacological action. This provided definitive evidence that the stereospecific binding site previously described were, in fact, opiate receptors.

DISTRIBUTION OF THE OPIATE RECEPTOR

The existence of opiate receptors was thus established, while nearly simultaneously, work was under way to examine the regional distribution of opiate receptors within the nervous system. In their initial study describing the opiate receptor, Pert and Snyder also examined the regional distribution of these receptors (54). In rats, they found the highest amount of binding in the corpus striatum, while the midbrain and cortex had only one-fourth this amount of binding. A more detailed study was then done using ^3H -diprenorphine in monkey and human brains (40). This study revealed high levels of binding in the amygdala, periaqueductal area of the midbrain, hypothalamus, and medial thalamus, while levels of binding in white matter areas was low or undetectable. Another study, using homogenates of human brain and ^3H -etorphine, found similar results with the highest degree of binding in components of the limbic system (31).

All of these initial studies utilized regional dissection of tissues followed by homogenization as a means of investigating the distribution of receptors, and were thus limited in their degrees of anatomical resolution attainable. Therefore, investigators turned to microscopic techniques involving autoradiography in order to gain more precision in mapping out the opiate receptors.

Pert et al. (52, 53) investigated opiate receptor distribution in rats using light microscopic autoradiography. Brain and spinal cord tissue was extracted one hour following intravenous injection of ^3H -diprenorphine, and autoradiographs were prepared using a technique which minimized translocation of the radioactive ligand during preparation of the radiographs. The autoradiographs revealed areas of high grain density restricted to relatively small areas, including the caudate-putamen, locus caeruleus, and the substantia gelatinosa of the spinal cord.

Similar results were found in a detailed study of the spinal cord and medulla of rats by Atweh and Kuhar (1), using ^3H -diprenorphine and ^3H -etorphine. In addition to laminae I and II of the spinal cord, the autoradiographs showed high grain densities in

the substantia gelatinosa of the spinal trigeminal nucleus, components of the vagal system, and the area postrema.

These studies made it apparent that opiate receptors were distributed in areas thought to be important in relaying, processing, or modulating nociceptive information, and in other areas which may be involved in mediating known non-analgesic effects of opiates: e.g., area postrema--nausea/vomiting, solitary nuclei--depression of cough reflex, and limbic system--euphoria.

In examining such "maps" of opiate receptors, it was also noted that the receptors were distributed in a manner closely associated with the paleospinothalamic pain pathway (61). The paleospinothalamic pathway ascends along the midline of the brain, passing through the periaqueductal grey, medial thalamus, and parts of the limbic system, and projects diffusely onto large areas of the cortex. This system is thought to be involved in the transmission of dull, poorly localized pain. This is in contrast to the neospinothalamic pain pathway, which is more laterally located, and projects to the somatasensory cortex via the ventral posterolateral nuclei in the thalamus. This system is thought to have evolved later, and is involved in

the transmission of sharp, localized pain.

This close association of opiate receptors with the paleospinothalamic systems helps explain the clinical characteristics of opiate analgesia. That is, the effective control of dull, poorly localized pain and a modulation of the "emotional component" of pain (through effects on the limbic system), and relatively poor control of sharp, localized pain.

It should be mentioned briefly that attempts have been made to carry the localization of opiate receptors one step further and determine whether they are located pre- or post-synaptically with respect to the primary afferent fibers in the spinal cord. Evidence exists for both a pre- and post-synaptic location of opiate receptors (76, 77). A study by LaMotte and co-workers (42) showed a significant reduction in stereospecific opiate binding in the dorsal horn of the spinal cord of monkeys following rhizotomies, suggesting a pre-synaptic location of the opiate receptors. The reduction in binding was, nonetheless, subtotal, implying that a portion of the receptors may exist post-synaptically. Although this area remains somewhat unclear, it appears likely that pre- and post-synaptic receptors exist, and that both sites can be acted upon by exogenously applied opiates to suppress nociceptive messages.

THE ENDOGENOUS OPIOIDS

Even as the distribution of opiate receptors was being elucidated, investigators were intrigued by the presence of highly specific receptors in vertebrate tissues which interacted with derivatives of the poppy plant. The existence of these receptors suggested the presence of a natural, endogenous "morphine-like" substance, and, as expected, a search led to the discovery of such a substance.

Terenius and Wahlstrom (66) found a purified extract from rat brain which was capable of inhibiting the binding of dihydromorphine to receptors. The solubility characteristics of this extract were suggestive of the presence of an oligopeptide.

Hughes (34) had independently isolated a low molecular weight morphine-like substance from rabbit, guinea-pig, rat, and pig brain which inhibited electrically evoked contractions in the mouse vas deferens and guinea-pig ileum (like the guinea-pig ileum, the mouse vas deferens contains stereospecific opiate receptors which mediate neurally evoked contractions). This inhibition was reversed with naloxone. Hughes named this substance naloxone-reversible-activity (NRA), and suggested that it was a peptide or peptide containing molecule of

molecular weight less than 700. Pasternak (51) had also isolated a similar morphine-like factor (MLF) from calf and rat brain.

Hughes went on to identify NRA from pig brain and found that it was composed of two pentapeptides, which he called methionine-enkephalin and leucine-enkephalin (35) [enkephalin = Gk. "in the head"].

Earlier in the same year, Goldstein and his colleagues had isolated a morphine-like substance from bovine pituitary glands (12,67) which was apparently distinct from the enkephalins described by Hughes. In addition, Hughes had noticed the peculiar fact that the sequence of methionine-enkephalin was identical to that of residues 61-65 of β -lipotropin, a 99 amino acid pituitary peptide involved in fat breakdown (35). Li, who had isolated β -lipotropin, had also isolated a 31 amino acid fragment of β -lipotropin from camel pituitary whose function was unclear (44). Putting all of this together, investigators (11,45) were able to show that this 31 amino acid fragment of β -lipotropin from the pituitary had potent opioid activity, and named it β -endorphin (from ENDogenous mORPHINE). This accounted for only part of the opioid activity in Goldstein's pituitary extract. Another distinct component was found in the extracts

with potent opioid activity and named dynorphin (23).

Thereafter, a variety of other peptides with opioid activity were isolated from pituitary and brain tissue (10).

Although it was initially speculated that β -lipotropin or its fragments might have served as precursors to enkephalin, this is now thought not to be the case (see 63). In fact, the endogenous opioid peptides have now been separated into three general classes, where members of each class are derived from a different precursor (63):

<u>CLASS</u>	<u>PRECURSOR</u>
ENKEPHALINS-----	PREPROENKEPHALIN
ENDORPHINS-----	PREPROOPiomelanocortin
DYNORPHINS-----	PREPRODYNORPHIN

The exact functions of each of these endogenous opioids are not entirely clear. The regional distribution of enkephalins closely parallels that of opiate receptors, and they are apparently localized in nerve endings, suggesting that they may be neurotransmitters involved in mediating sensory information concerned with pain and emotional behavior. Enkephalins have also been found in the small intestine of a number of species indicating a possible role in gastrointestinal motility (25). β -endorphin is found predominantly in the posterior pituitary and hypothalamus, and is essentially absent

in the remainder of the brain (63). Thus, it has been suggested that β -endorphin may play a role in modulating pituitary activity, such as release of antidiuretic hormone (63), and that its analgesic action may be unrelated to its normal physiological function (61). There is evidence, however, that β -endorphin may be involved in pain modulation (33). The function of the dynorphins is even less well understood.

Although the details of their respective functions remains to be determined, methionine-enkephalin, leucine-enkephalin, and dynorphin have been found in the dorsal horn of the spinal cord (10, see below).

RECEPTOR SUBTYPES

As these various endogenous opioid compounds were being isolated and characterized, it became evident that there may actually be more than one type of opiate receptor. Martin (48) described three distinct behavioral syndromes produced by various opiate agonists in chronic spinal dogs, and attributed each of these to effects at separate opiate receptor subtypes, which he named mu (u), kappa (k), and sigma(σ). Since this initial study, other opiate receptor subtypes have been described, including delta (d) and epsilon (e) (47,74)

SUBTYPES WITHIN THE SPINAL CORD

A detailed description of all the subtypes, their distribution, and the effects which they mediate is beyond the scope of this discussion. It should be mentioned, however, that within the spinal cord, the presence of u-, k-, and d- receptors has been described (19,24,70,71). Although the relative importance of these receptors in nociception is not clear, a study by Schmauss and Yaksh (58) has provided evidence that these receptor subtypes may be involved in modulating different types of nociceptive stimuli. They found that in rats, intrathecal administration of mu and delta, but not kappa, agonists inhibited responses

to cutaneous thermal stimuli, while mu and kappa, but not delta, agonists inhibited responses to visceral chemical stimuli. This is part of the evidence available which suggests that there may be distinct endogenous opioid systems at the spinal cord level which subserve the processing of different types of nociceptive input.

SITES OF OPIOID ACTION

The sites and modes of action of opioids has been an area of much speculation for many years. The identification of the opiate receptor and discovery of the endogenous opioid peptides has helped immensely in the understanding of this area, although in some ways (e.g., the concept of multiple opiate receptor subtypes) it seems to have added complexity to an already confusing field.

One area in which a significant amount of knowledge has been gained is that of opioids and their effects within the spinal cord. As may be expected, the literature on this subject is already quite voluminous; an attempt will be made here to review only those investigations felt to be most relevant to the present study.

SPINAL VS. SUPRASPINAL ACTION OF OPIOIDS

There has been, and perhaps still is, some controversy regarding the effects of opioids at the spinal level. In many cases, earlier investigators favored the view that systemically administered opioids activated descending pathways supraspinally which then inhibited transmission of nociceptive information at the spinal level, rather than a direct effect at that level.

Basbaum and Fields (2,18) described a brainstem analgesic

system involving the nucleus raphe magnus in the medulla, and speculated that projections from this supraspinal site to the spinal cord were essential to "opiate-produced analgesia."

A study by Satoh and Takagi (57) showed that intravenous morphine decreased the amplitude of evoked potentials recorded from the ventrolateral fasciculus of the spinal cord following stimulation of the splanchnic nerve in pentobarbital-anesthetized cats. This decrease in amplitude was reversed when the spinal cord was blocked at the C-1 level, leading the investigators to conclude that the observed effect was due to an increased descending inhibition of spinal sensory transmission. Duggan has pointed out, however, that tonic descending inhibition may be present in barbiturate-anesthetized cats, making the results of Satoh's study difficult to interpret (16). Nevertheless, other investigators also felt that the evidence available at the time supported "activation of a descending inhibitory system" as the mechanism responsible for opioid analgesic action (28,50).

Other studies, however, were beginning to show that opioids may have a direct effect at the spinal cord level, independent of its effects at supraspinal

sites. Evidence that opioids may have significant effects at the spinal cord level was, in fact, presented over forty years ago by Wikler (73). Using acute and chronic spinal cat preparations, Wikler found that intravenous morphine (2-15 mg/kg) markedly depressed responses to nociceptive stimuli (flexor and crossed extensor reflexes) while responses to stretch (knee and ankle jerks) were unchanged or slightly enhanced. This suggested the possibility that morphine was having a direct effect upon interneurons within the spinal cord.

Nearly ten years later, Koll et al. (39) showed that ipsilateral nociceptive "post-delta-flexion" and "C-flexion" reflexes were suppressed by analgesic doses of morphine (0.3-0.4 mg/kg) in decerebrate, spinal cats.

These studies, while suggestive of an action at the spinal level, were somewhat indirect in that motor responses, which may not necessarily be related to an analgesic effect, were examined.

In order to study more directly the effects of opioids on spinal systems thought to be involved in pain, Besson et al. (3) examined the electrophysiological

effects of phenoperidine on dorsal horn lamina V cells in spinal cats. Lamina V cells were studied due to their apparent importance in the transmission and integration of nociceptive information. Extracellular unitary recordings of cell activity showed that intravenous phenoperidine (0.2 mg/kg) markedly reduced both spontaneous and evoked activity in the majority of cells studied. These effects were reversed by intravenous nalorphine. Thus it appeared that phenoperidine had a direct effect on cells involved in pain transmission at the spinal cord level. It was not clear from this study, however, whether this effect was specific to lamina V cells.

Kitahata et al. (38) were the first to demonstrate such a lamina-specific suppressive effect of morphine. Using extracellular recordings in decerebrate, spinal cats, they were able to show a dose-dependent suppression of spontaneous and evoked activity of cells in laminae I and V (associated with nociception) by morphine (0.5-2.0 mg/kg i.v.). Activity of cells in laminae IV and VI, which respond to non-noxious cutaneous stimuli and proprioceptive stimuli, respectively, was unaffected. Thus, morphine was shown to have a *selective* effect on cells involved in nociception at the spinal level.

Lebars et al. (43) found similar results in a study looking only at lamina V cells and using a single dose (2 mg/kg i.v.) of morphine. They were also able to show that the suppressive effects of morphine on spontaneous and evoked activity were reversed by naloxone or nalorphine, implying a specific, receptor-mediated action.

Calvillo et al. (6) showed that microiontophoretically applied morphine had a depressant effect on dorsal horn neurons which responded to noxious radiant heat applied to the skin, but had very little effect on neurons responding to non-noxious stimuli. This effect was reversed by both iontophoretically and intravenously administered naloxone.

Other studies also showed a direct spinal action of opioids (14,68,69).

It therefore appeared that opioids were capable of exerting a direct effect at the spinal level on nociceptive cells. From the above studies, however, it was not evident if the observed effects actually translated into analgesia in the intact organism.

Yaksh and Rudy (78) answered this question and went a step further by examining the effects of opioids administered directly into the spinal subarachnoid space of intact animals. Using rats with a chronically implanted catheter in the spinal subarachnoid space, they assessed the effects of opioids (fentanyl, morphine, codeine, ethylmorphine) on pain thresholds as tested by the tail flick response (spinally mediated), and the hot plate and squeak-escape responses (both responses with a supraspinal component). Small volumes (5 μ l) of drug injected through the catheter elevated the thresholds in all tests in a dose related manner, and their effects were reversed by both intraperitoneal and intrathecal naloxone. The possibility of rostral spread of the injected drug or redistribution to other sites via the bloodstream were both effectively excluded. In a similar manner, Yaksh (75) showed that small doses (40-160 μ g) of intrathecal morphine produced behaviorally defined analgesia of prolonged duration (greater than 10 hours with 80 μ g) in the cat and monkey.

RELATIVE SIGNIFICANCE OF SPINAL AND SUPRASPINAL EFFECTS

Thus, it was shown that opioids, acting directly at the spinal cord level, could produce analgesia in the intact animal. A question which remained was, how much, if any, of the analgesic action of systemically

administered opioids was attributable to an effect at the spinal level relative to supraspinal sites of action? Using a similar preparation as above, Yaksh and Rudy (79) found that following intraperitoneal administration of an analgesic dose of morphine, intrathecal naloxone (up to 40 ug) was not able to completely reverse the analgesic effects. This suggested that the morphine was acting upon spinal sites and supraspinal sites; the latter site not being accessible to the intrathecally administered naloxone.

Hanaoka et al. (27), in another study, compared the effects of intravenous morphine on the activity of lamina V spinal neurons in cats with intact and transected spinal cords. They found a greater degree of suppression of spontaneous and evoked activity of these cells in cats with intact spinal cords as compared to those with the cord transected. They also found, however, that intravenous naloxone was able to reverse this suppressive effect in cats with transected spinal cords, leading them to conclude that there was a direct effect at the spinal cord level, which may be modulated to some degree by a supraspinal effect.

It seems, therefore, that for systemically administered opioids, effects may be present at both supraspinal

and spinal sites, giving rise to analgesia in the intact animal (as noted above, however, there is still some controversy regarding this issue--see 7,8).

For the sake of completeness, it should be noted that opioids are thought not to affect conduction through peripheral nerves--another possible site of action (41,77,81).

SITES OF OPIOID ACTION WITHIN THE SPINAL CORD

One aspect of spinal opioid analgesia which remains to be clarified--something with which this study is concerned--is that of the site of opioid action within the spinal cord itself. Attention has focused on the dorsal horn, as several lines of evidence point to this area as the site of action of spinal opioids.

Duggan et al. (15) showed that iontophoretic administration of morphine into the substantia gelatinosa region of the dorsal horn inhibited the discharge of WDR neurons in laminae IV and V in response to nociceptive stimuli, while responses to innocuous stimuli were unchanged. These effects were reversed by naloxone given iontophoretically into the substantia gelatinosa or given intravenously. In contrast to this, when morphine was administered in the vicinity of the cell bodies of the neurons,

no such inhibitory effect was observed. This suggested that opioids were acting upon receptors in the upper part of the dorsal horn--namely, the substantia gelatinosa. As noted earlier, this is an area where autoradiographic studies have demonstrated a relatively high concentration of receptors.

Further evidence that the opioids may be acting in the dorsal horn comes from comparing the time course of inhibition of lamina V WDR neurons following intrathecal morphine with that of the appearance of behaviorally defined analgesia after intrathecal morphine. Onset of the anti-nociceptive effect of intrathecal morphine (80-500 ug) when measured by the thermally evoked skin twitch is at around 15 to 20 minutes in cats (76). Similarly, spinally applied morphine (250 ug) reduces the spontaneous and noxiously evoked activity of lamina V WDR neurons in decerebrate, spinal cats by approximately 70% within 25 minutes (32). Therefore, at a time when nociceptive cells in the dorsal horn are inhibited, analgesia is present, again suggesting an action in the dorsal horn.

It has also been shown that spinally applied Fentanyl (25 ug) will inhibit the noxiously evoked activity of dorsal horn WDR neurons by about 70% six minutes

after application, as compared to the 25-30 minutes required for morphine to achieve the same degree of suppression (64). This faster action of fentanyl may be related to its lipid partition coefficient, which has been correlated with the rate at which these drugs diffuse into brain tissue (30). Thus, it has been speculated that fentanyl's lipophilicity allows it to penetrate relatively quickly to the site of action in the dorsal horn, while morphine's poor lipid solubility hampers its penetration into the dorsal horn, delaying the onset of its action.

Such lines of evidence suggest that the dorsal horn is a probable site of action of spinally administered opioids. Duggan's work (above) further implicates the substantia gelatinosa region as a possible site of action. There are no studies, however, which show what the rate and extent of penetration of spinally administered opioids into the spinal cord are. For example, while iontophoretically applied opioids in the substantia gelatinosa may be able to inhibit the activity of nociceptive cells, we do not know if these drugs, when given spinally, are actually able to reach these potential sites of action within an amount of time consistent with the electrophysiological studies (32,64). The current study hopes to clarify this issue.

CLINICAL USE OF SPINALLY ADMINISTERED OPIOIDS

The potential spinal action of opioids has attracted considerable clinical interest. As mentioned above, animal studies showed that opioids applied in small doses directly to the spinal cord were able to produce a behaviorally defined analgesia of long duration. In addition, this analgesia was produced without any apparent effects on motor, respiratory, or autonomic functions of the animal (75). These facts, together with the already established techniques for administering drugs into the spinal subarachnoid and epidural spaces, suggested exciting possibilities for the control of pain in clinical settings. It appeared ideal, for example, for the control of postoperative pain--pain relief without any motor, respiratory, or autonomic deficits.

Although intrathecal morphine may have been used clinically over 80 years ago (49), Wang and his colleagues (72) in 1979, were the first to report on a double-blind, controlled study of intrathecal opioids in humans. In six out of eight patients suffering from intractable pain secondary to cancer, intrathecal morphine (0.5-1.0 mg) produced complete pain relief for 12 to 24 hours. The remaining two patients reported pain relief after morphine and after saline injection, although

the relief lasted longer after morphine. The investigators found no evidence of sedation, respiratory depression, or impairment of other neurological functions.

This report was soon followed by many other clinical studies on intrathecal and epidural administration of opioids (see 9), prompting the statement that "never in the history of medicine has a concept progressed as rapidly from laboratory experimentation with animals to clinical application in man...(17)"

This initial enthusiasm was somewhat tempered by reports of various side effects of intrathecal and epidural opioids, including pruritis, nausea, vomiting, and, most importantly, delayed respiratory depression.

Nonetheless, clinical studies using spinal opioids have become increasingly common. Intrathecal and epidural opioids have been shown to be effective in management of postoperative and cancer pain, while their efficacy in controlling the pain of labor remains controversial (9,76). Epidural opioids have received considerably more attention in these investigations, perhaps in part due to the lower incidence of respiratory depression associated with this route of administration (9,26) and also to the relative ease of giving repeated doses.

Methods using indwelling catheters for repeated intrathecal or epidural administration of opioids and totally implantable systems have been used successfully in some cases for the treatment of chronic pain states (9,76).

Thus, epidural and intrathecal opioids hold much promise as new methods of managing acute and chronic pain. Data from animal and human studies, however, are still incomplete. Bromage (4) pointed out early on the need for extensive basic and clinical studies and cautioned against the widespread use of these techniques without due consideration for the side effects produced. Despite continuing accumulation of data from basic and clinical investigations, it is not yet known which drugs in what doses and in what volumes should be used. Thus, spinal administration of opioids in humans is best considered as still being in an investigational stage at this time. As further knowledge on the relevant anatomy, physiology, and pharmacology of spinal opioids is gained, safe and appropriate use of this technique should be possible.

CURRENT STUDY

AUTORADIOGRAPHIC TECHNIQUE

As described above, various methods have been employed in attempts to study opiate receptor localization and opiate binding, beginning with tissue homogenates and proceeding to autoradiographic methods. Autoradiographs are based on the principle that ionising radiation from radioactive isotopes in a sample can induce a change in silver bromide crystals suspended in the adjacent emulsion such that when the crystal is exposed to a developing agent, the crystal is converted to metallic silver. A fixative solution then dissolves out the crystals not reduced to metallic silver, leaving a pattern of silver grains which reflect the distribution of radioactive material in the underlying sample. This study uses tritium as the radioactive isotope. Tritium decays by emitting B particles (essentially electrons of nuclear origin) and has a half life of 12.3 years.

The first autoradiograph is said to have been made in 1867 (55). Many refinements and modifications in the technique have been made since that time. In the case of autoradiographs involving diffusible materials, (as in the present study) one technical difficulty is encountered in trying to limit diffusion of the

radioactive particles during the various phases of tissue processing. Several techniques have been developed in an attempt to create radiographs which reflect the true distribution of radioactive molecules (55, 56, 20). Although slight differences exist in these various techniques, most depend on the use of rapidly frozen tissue which is allowed to dry at low temperatures in low humidity environments. Such techniques were used by Atweh and Kuhar (1) in their studies on the anatomical distribution of opiate receptors referred to above. More recently, Herkenham and Pert (29) described a technique in which tissues were fixed using dry formaldehyde vapors prior to processing, resulting in well preserved tissue quality without disruption of specific opiate binding.

Regardless of the specific technique used, autoradiography is felt to be particularly suited for receptor and ligand distribution studies as it allows the visualization of relatively low concentrations of labelled drugs and, in addition, allows for localization of the drug within intact tissue, rather than homogenates or otherwise anatomically disrupted tissues. This makes it possible to correlate anatomical/structural data with pharmacological and physiological data--something this study hopes to do (see below).

The autoradiographic technique used in the current study attempts to minimize redistribution of the labeled molecules during tissue processing. However, compared to some more rigorous methods, significant movement of labeled drug is thought to occur. The degree of such movement, however, is felt to be important only if localization is attempted at the cellular level. This preliminary study is concerned only with the overall distribution of the drug--not specific binding--and thus the technique used is felt to be satisfactory. As studies requiring detailed localization are performed, more rigorous methods will undoubtedly need to be employed.

AIMS:

Before proceeding further, it should be noted and stressed that the current study is of a preliminary nature. Aside from the aims discussed below, one major goal is to establish the experimental technique necessary for more detailed studies in the future. Thus, it is expected that some variations in the methodology will occur during the study, and that the initial sets of data obtained may not necessarily lead to definitive conclusions.

That notwithstanding, one objective of this study is to investigate the nature of penetration of spinally

applied morphine into the spinal cord tissue. By applying radiolabelled morphine and obtaining autoradiographs of spinal cord tissue at specified time intervals, an indication of the depth of penetration and distribution of spinally administered morphine in the spinal cord is hoped to be obtained.

Furthermore, the data obtained on the penetration of morphine will be compared to existing electrophysiological data on spinally administered morphine. In particular, attempts will be made to relate this study's findings with those in a study by Homma et al. (32).

Homma examined the effects of spinally applied morphine (0.1 & 0.25 mg) on the activity of dorsal horn WDR neurons of decerebrate, spinal cord-transected cats. It was found that 0.25 mg of morphine applied directly onto the spinal cord was able to suppress both spontaneous and noxiously evoked (radiant heat) activity of WDR neurons. The data of interest is shown in figure 2, which illustrates the time course of suppression of the WDR neurons. At 15 minutes, evoked activity is suppressed to 48% of control, and at 30 minutes, it is suppressed to 30% of control values. In several neurons followed for one hour after morphine administration, there was no indication of recovery of neuronal activity.

These effects were partially reversed by intrathecal naloxone and further reversed when intravenous naloxone was added.

In the current study, a relationship between the temporal changes in drug localization (as indicated by the autoradiographs) and physiological responses to the drug (as indicated by Homma's study) will be sought. More specifically, an attempt will be made to show whether the reduction in WDR activity described above depends on the availability of morphine in that region, or is independent of it.

In addition, some indication of the speed of penetration of morphine into the spinal cord is hoped to be obtained. The relatively slow onset of spinal morphine analgesia, as noted previously, has been ascribed to its low lipophilicity which has been thought to be a limiting factor for penetration of the drug to its sites of action within the cord matter. Is this in fact the case--i.e., can the slow onset of action of spinal morphine be explained by slow penetration of the drug?

This study is not constructed to allow any inferences about specific opiate binding. The autoradiographs will only show overall distribution of morphine within

the cord, and will include specifically and nonspecifically bound molecules which will not be distinguishable. It is therefore not the intention of the current study to define a precise site of action of spinally applied morphine within the spinal cord.

In summary, the objectives of this study are to:

1. Begin to establish an appropriate autoradiographic technique for the study of penetration of opioid compounds into the spinal cord for use in future studies.
2. Obtain an indication of the rapidity with which morphine penetrates into the spinal cord following spinal administration and relate this to the known delay in onset of spinal morphine analgesia.
3. Seek a relationship between temporal changes in drug localization and known physiological responses of dorsal horn WDR neurons.

METHODS

Experiments were conducted using adult cats of either sex weighing approximately 3-4 kg. Animals were anesthetized with a mixture of halothane, nitrous oxide, and oxygen. Cannulations of an external jugular vein and a carotid artery were then performed, followed by a tracheostomy. The animals were paralyzed with pancuronium bromide and respirations were controlled by a positive-pressure ventilator. The animal was then placed in a stereotaxic frame and decerebrated by bilateral electrolytic lesions in the midbrain reticular formation. Anesthesia was then discontinued, and ventilation was performed with 100% oxygen. Arterial blood pressure, core body temperature, and end-tidal CO₂ were monitored and maintained within normal limits. Laminectomy was then performed at L1 through S1 and the dura was reflected, exposing the spinal cord. Warm normal saline was placed onto the cord as necessary to prevent it from drying out.

The morphine solution to be applied was then made, using 25 ul of ³H-morphine (72.7 Ci/mmol, 1 mCi/cc, New England Nuclear) and 0.25 mg of morphine sulfate in 0.5 cc of normal saline. The resulting solution (total volume of 525 ul) was applied directly onto the central portion of the exposed cord following gentle

suctioning of the fluid surrounding the cord.

Following a time interval of 30 seconds, 5 minutes, or 10 minutes, the cord was rapidly dissected out, dipped quickly into chilled normal saline, then immersed in isopentane cooled to approximately -65°C with dry ice. After 30 seconds, the frozen cord was removed and cut into five sections (numbered 1-5 from rostral to caudal) each 2-3mm thick, which were replaced into the cooled isopentane. The time required for cord removal varied slightly, but averaged around 60 seconds. In some cases, the solution covering the cord was suctioned off immediately before cord removal.

Individual pieces of the frozen tissue were affixed to tissue chucks using an embedding compound (Lipshaw M-1) and sectioned using IEC-CTF cryostat (International Equipment Corp.) at -15°C. Section thickness was set at 8 μ . The tissue sections were picked up onto cold standard microscope slides which had been previously cleaned and coated with gelatin. Slides were then placed into a slide box with dessicant capsules and kept under vacuum in a dessicator at -15°C until further processing.

The next step involved dipping of the slides into a liquid nuclear emulsion. Under appropriate safelighting conditions, slides which had been allowed to warm to room temperature under vacuum were dipped vertically into a liquid emulsion (Ilford K-5) diluted 1:2 with double distilled deionized water which was kept at 40°C in a water bath. The dipped slides were then allowed to drain and dry partially for about 15 minutes in a vertical position on a slide rack to ensure a consistent thickness of the emulsion over the tissue sections. The slides were then transferred to light tight slide boxes with dessicant capsules, and stored under vacuum in a dessicator at -15°C for exposure.

Following exposure under those conditions for four weeks, the slides were allowed to warm to room temperature once again under vacuum in the dessicator in preparation for development. Development was carried out under appropriate safelighting using Kodak D-19 developer, Kodak Indicator Stop Bath, and Kodak Fixer. Slides were developed for 3 minutes at 20°C, with 1 minute in the stop bath, 5 minutes in the fixer and two rinses in distilled water.

The developed slides were then stained using a 2.5% cresyl violet solution. Slides were immersed

directly into the cresyl violet after the development process for 3-5 minutes then subsequently immersed in distilled water, 75% ethanol, 90% ethanol, and 100% ethanol for approximately 30 seconds each.

Autoradiographs prepared in this manner were then observed under a microscope (Olympus BH-T). Counting of autoradiographic grains was done under direct visualization by counting within a $625 \mu^2$ area as outlined by a grid superimposed upon the tissue image by a camera lucida attachment. Sample grain counts were obtained from the posterior column area, laminae I, II, V, VII, and the ventral horn area (figure 3). For each area of tissue sampled, three separate, randomly selected areas were counted, and the average of these was taken as the grain count for that area.

For each time interval, tissue was obtained from two animals. Statistical analyses were carried out using the t-test for unpaired data.

It should be noted that the technique described above was arrived at after a variety of problems were encountered and several modifications introduced.

Tissue was initially sectioned at a 16 μ thickness, but it became evident that thinner sections would be required to allow even staining and prevent extensive overlapping of autoradiographic grains. Emulsion thickness was also a problem--attempts to use undiluted emulsion led to radiographs with several levels of exposed grains superimposed upon each other making counting quite difficult. Experiments with several different dilutions showed that the 1:2 dilution provided a uniform and nearly single layer of grains. One of the most crucial aspects in terms of preservation of histological quality was the temperature of tissue. During the initial studies, slides were often allowed to warm to room temperature then replaced into the freezer several times prior to final development and staining. This often led to wrinkled and cracked tissue of poor histological quality. Subsequent experiments were carried out in such a manner as to minimize the number of times which the tissue was allowed to warm to room temperature--in the case of the most recent studies, this was reduced to twice: when the slides are dipped into emulsion and prior to development. This led to a remarkable improvement in histological

quality. Modifications continue to be made at this time in attempts to improve the consistency and quality of the autoradiographs.

RESULTS

Using the methods described above, autoradiographs of cross sections of the spinal cord were obtained which had good histological preservation and uniformly low radiographic background (in general 0-2 grains/625 μ^2). Control slides prepared with "cold" tissue showed no evidence of chemographic artifacts. Figure 4 shows representative photomicrographs of the exposed, developed and stained autoradiographs.

Tables 1, 2, and 3 show the mean grain counts per 625 μ^2 for each of the areas sampled, listed according to the "segment" from which the tissue came, for the 30 second, 5 minute, and 10 minute intervals, respectively. It was initially expected that grain counts would show a rostral-caudal difference in distribution which would be evident as differences between counts in segments 1-5. Inspection of tables 1, 2, and 3, however, does not reveal any consistent trend in grain counts by segments.

Thus, data was also pooled without regard to segment number, to obtain mean counts of the "total" for each area sampled. These results are shown in table 4, and graphically represented in Figure 5.

For laminae V, VII, and the ventral horn, there was a statistically significant *decrease* in the mean grain density from 30 seconds to 5 minutes. The remainder of the areas showed no significant changes in grain density during that interval. From 5 minutes to 10 minutes, however, all areas showed a significant increase in mean grain density (fig. 5).

DISCUSSION

One aim of this study was to establish an autoradiographic technique for the study of penetration of opioids into the spinal cord. The technique developed in the current study appears to be useful for this purpose. That is, it was possible, using the technique described above, to "follow" the penetration of spinally applied morphine into the spinal cord with time. Nonetheless, some problems do remain. If these autoradiographs were, in fact, representative of the distribution of morphine molecules at given moments in time, one might expect a relatively clear "front" of activity of the penetrating drug. No such clearly demarcated "fronts" of activity were seen, however, in any of the tissue samples. Also of note is the relatively large variability in the grain counts (see tables 1-3).

These observations suggest the possibility of significant movement or diffusion of the morphine during processing. Although the technique employed attempted to minimize such movement, a few of the steps in processing may still be allowing for such movement to take place. During sectioning of the tissue on the microtome, the tissue will thaw to some extent as it is cut and carried across the blade. This thawing together with any ambient

moisture may have allowed for some translocation of the morphine molecules. It is also possible that, after repeated sectioning, the blade itself had become contaminated with radiolabelled morphine molecules which were then picked up randomly onto tissue sections as they passed over the blade. It is unlikely, however, that this contributed greatly to variation in grain counts. One final step during which movement of radiolabelled morphine may have occurred is the dipping of slides into emulsion. The moisture and elevated temperature of the emulsion solution contacting the tissue may have allowed for some movement of the morphine.

Regardless of the cause, future modifications in technique will need to control movement of drug during processing to a greater extent, as more anatomical resolution is required. The method described by Herkenham and Pert involving partial fixation of tissues prior to fixation may be the best way to achieve such control. The use of microscope slides pre-dipped in emulsion is another possibility, although this introduces the significant technical difficulty of manipulating fragile tissue sections under dark, safelit conditions.

Despite this variability, the results of this study show that spinally applied morphine, although relatively hydrophilic, is able to penetrate to the deeper laminae of the spinal cord within a few minutes of application. It can only be said, though, that the morphine penetrates to such a degree, and no conclusion can be drawn regarding its actions in those areas. That is, the results show that morphine penetrates relatively rapidly into the spinal cord, but it is not clear if the amount which has penetrated is sufficient for it to lead to an effect. Clinically, the onset of spinal morphine analgesia may require 30 minutes to 1 hour. Thus, it may be the case that while these results suggest relatively rapid penetration of morphine into the cord, a longer interval of time must pass before sufficient amounts have penetrated deeply enough to lead to the onset of analgesia. Proof of this, of course, will require more precise, quantitative methods to be developed and employed.

It was initially expected that the autoradiographs might show a front of drug activity as mentioned above, which would advance with time. As already noted, this was not found, making correlation with physiological data somewhat more difficult than expected. Nonetheless, within 30 seconds, morphine was present in levels above

background in all the areas sampled. Grain counts for all areas sampled also increased significantly between 5 and 10 minutes. These results are consistent with those of Homma et al. referred to earlier (see fig. 2). Within 3 minutes after morphine application, evoked activity of dorsal horn WDR neurons had been suppressed to about 70% of control. At twelve minutes, the activity was suppressed to about 55% on control. The increase in grains at ten minutes is consistent with this increased suppression. This suggests that suppression of lamina V WDR neurons may be dependent on the presence of morphine in the lamina V area. Again, it should be stressed that this study does not imply anything about specific drug-receptor interactions, or a precise site of action of morphine.

In general, there appears to be two possible mechanisms by which spinal morphine may lead to suppression of lamina V WDR neuron activity and thus, presumably, lead to analgesia. One is that morphine acts in the area of the substantia gelatinosa on either receptors of inhibitory interneurons or of dendrites of deeper lying lamina V neurons projecting superficially. The other possibility is that morphine acts directly on the cell body of the WDR neuron in lamina V to suppress its activity.

The study by Duggan using micro-iontophoresis of opioids described above (19) strongly suggests that opioids acted within the substantia gelatinosa to produce their inhibitory effects on deeper lying nociceptive neurons. The relatively rapid penetration of morphine into the spinal cord as demonstrated in this study, however, suggests that morphine could be acting at deeper levels. The results of the present study, while still preliminary, imply that the time course of increasing grain counts in the superficial and deeper layers of the cord is consistent with the time course of WDR neuron suppression as shown earlier (32). Thus, the possibility cannot be ruled out, based on this study, that spinally applied morphine acts at deeper layers of the cord to bring about suppression of WDR neurons located in those layers.

This study examined only three time intervals. As more intervals are examined (15, 30 minutes, 1 hour, e.g.), better correlation between drug location and physiological effect should be able to be made. In particular, it would be of interest to examine the grain distribution after extended intervals of time such as 6, 12, or 24 hours. At these intervals, non-specifically bound molecules may have diffused away, leaving only specifically bound molecules. Furthermore,

Future studies should examine the penetration characteristics of more lipophilic drugs such as fentanyl, which are assumed to act more quickly due to their rapid penetration into the spinal cord.

One result which is difficult to explain in this study is that of the significant decrease in grain density in laminae V, VII, and the ventral horn area between 30 seconds and 5 minutes. There is no apparent satisfactory explanation for this finding, except to attribute it to chance variation, despite statistical significance.

Finally, one possible criticism of the study design might concern the conditions under which the morphine was applied. These conditions were somewhat "unnatural" in that the spinal cord was exposed for a considerable length along which the dura and its vessels were reflected or removed. Needless to say this is somewhat different from the conditions under which the drug is administered clinically. The penetration characteristics of morphine may be quite different when an intact dura is present together with a circulating pool of CSF. Thus, it may be useful to design studies in which the drug is administered through a needle puncture in an otherwise intact dura to more closely simulate the actual clinical

situation.

In summary:

1. The present study has tested a technique of autoradiography as a method of studying penetration of opioid drugs into the spinal cord, and found this technique to be satisfactory, although it is concluded that further refinements/modifications will be necessary.
2. Despite its hydrophilic nature, spinally applied morphine was shown to penetrate to the deeper laminae of the spinal cord within minutes. No definitive conclusions can be drawn, however, concerning the action of the drug at these layers.
3. Temporal changes in the density of autoradiographic grains was found to be consistent with the initial time course of physiological responses to spinal morphine. Again, no inferences can be made about specific opiate binding or action.
4. Future studies should include experiments with longer time intervals after morphine application, modifications in the autoradiographic technique to minimize movement of the drug molecules, use of other more lipid soluble opioid compounds, and, finally, an experimental design more closely paralleling the clinical situation.

	NONSATURABLE (trapped and dissolved)	NONSPECIFIC SATURABLE	STEREOSPECIFIC SATURABLE
A			
B			
C			

FIGURE 1.

Schematic representation of conditions used by Goldstein (see text). Solid symbols represent radioactive levorphanol while open symbols indicate a 100-fold excess of nonradioactive compounds: dextrorphan in "B" and levorphanol in "C". Modified from Goldstein et al. (22).

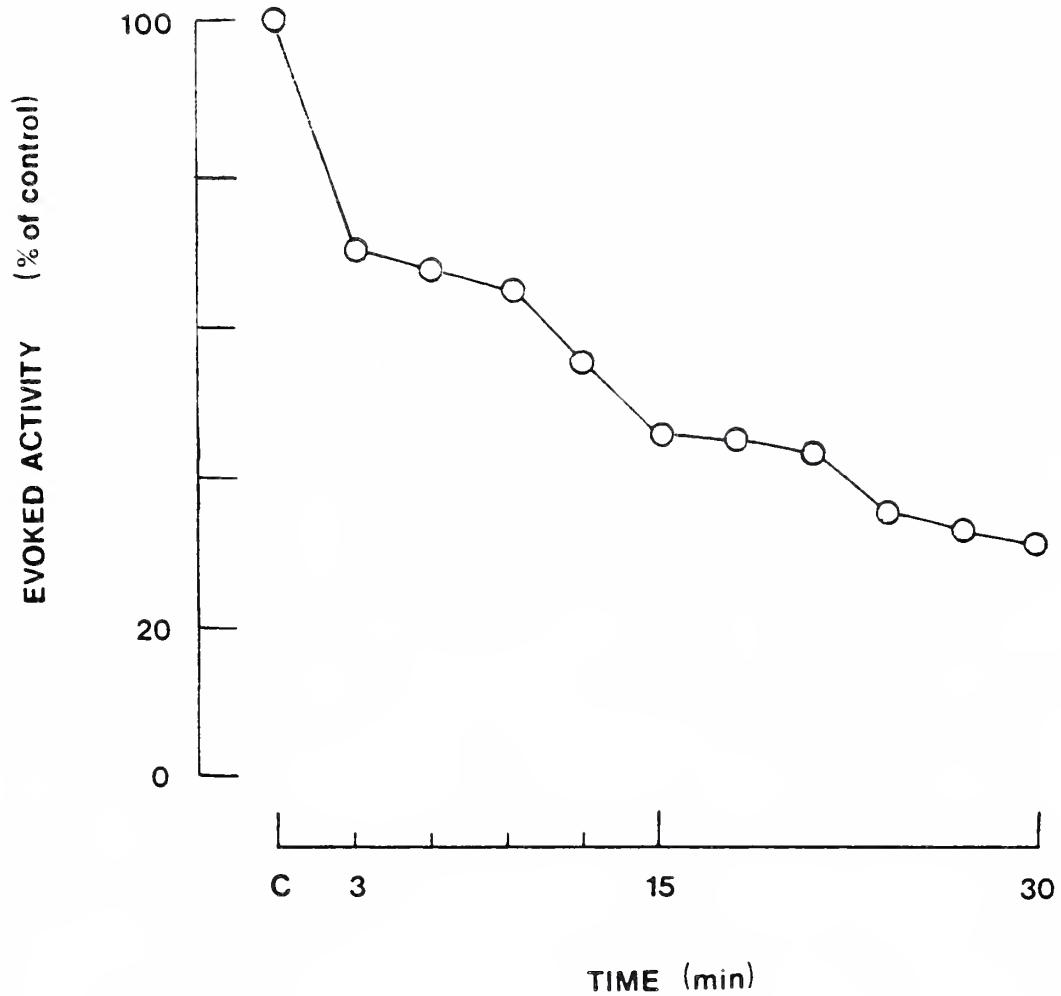


FIGURE 2.

Time course of suppression of dorsal horn WDR neuronal evoked activity following 0.25 mg of spinal morphine in cats. C = control. Modified from Homma et al. (32).

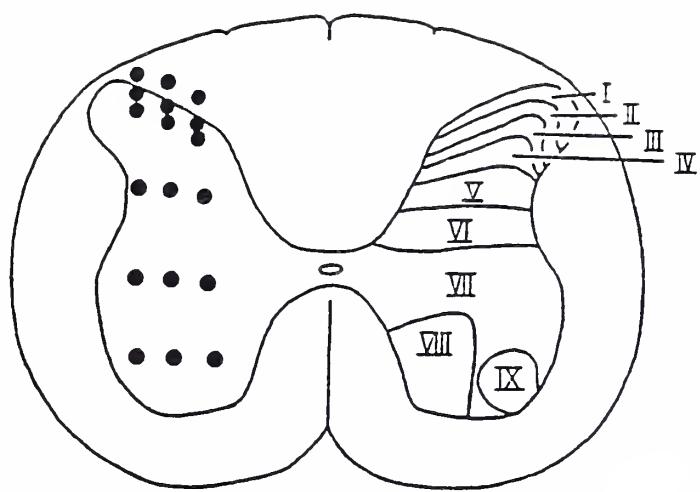


FIGURE 3.

Representation of areas in which sample grain counts were performed.
Each dot represents an area in which grains were counted.

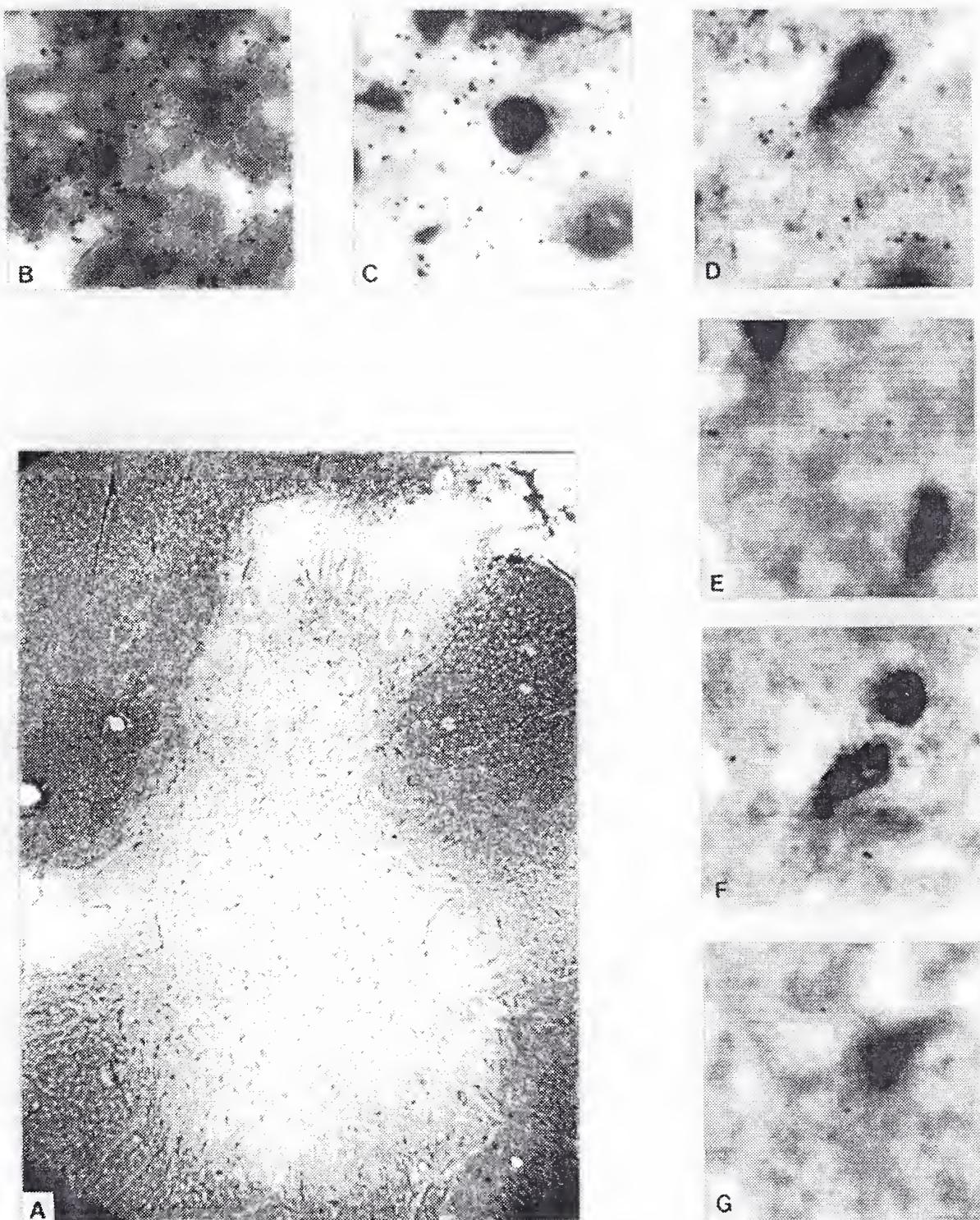


FIGURE 4 Low power view (mag. 20X) of autoradiograph of spinal cord cross section (A) with representative high power (mag. 1000X) photomicrographs of the various areas sampled. B ~ G taken at, respectively, posterior column, laminae I, II, V, VII, and ventral horn. Tissue shown was extracted five minutes after spinal morphine application. Focus is at level of the autoradiographic grains. Tissue stained with 2.5% cresyl violet.

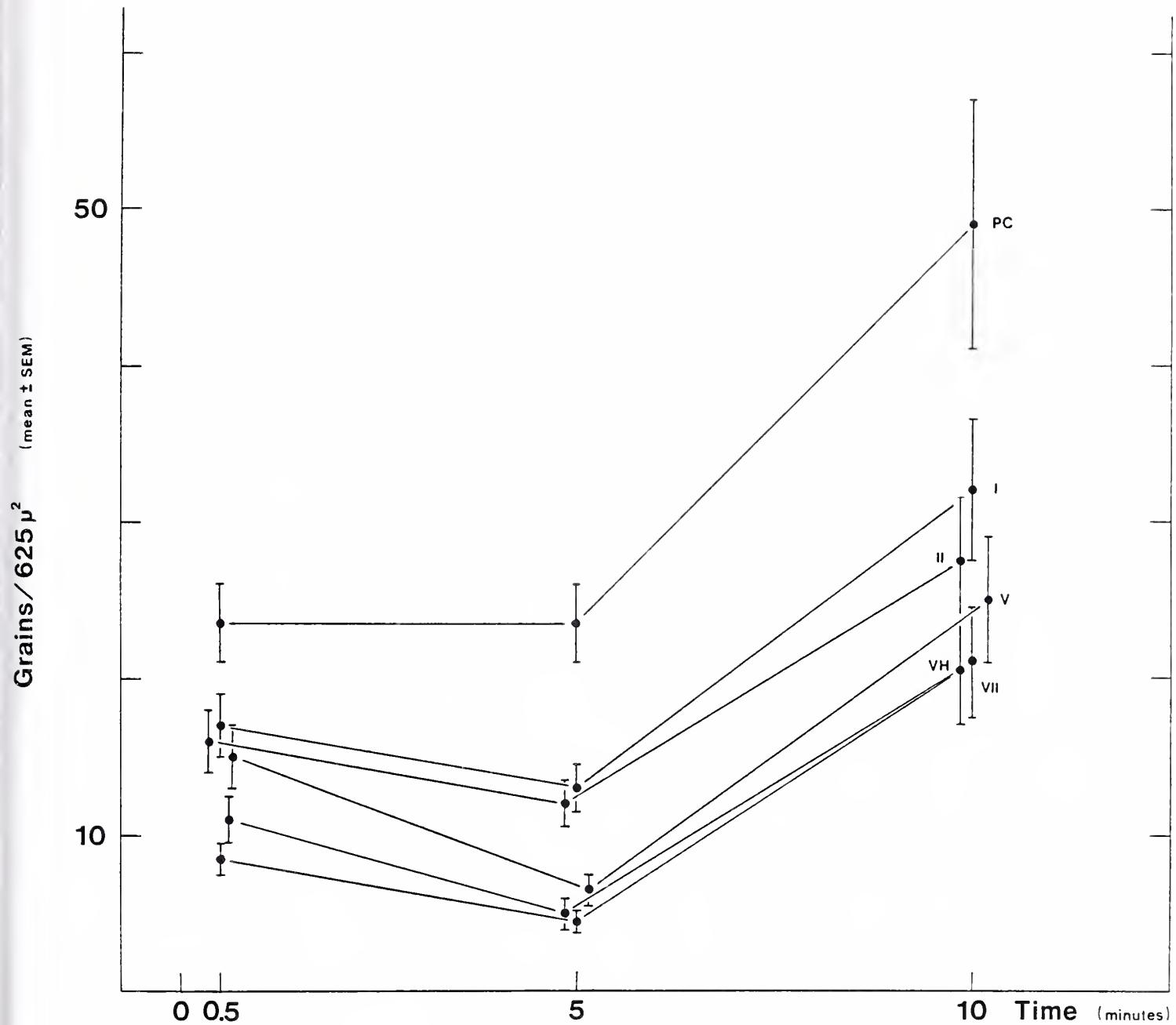


FIGURE 5.

Mean number of grains per $625 \mu^2$ for each time interval. Data derived from aggregate of values without regard to segment for each time. See also table 4.

GRAINS PER 625 u^2 (mean \pm SD) 30 seconds

SEGMENT	(n)	P.C.	I		II		V		VII		V.H.
			1	2	1	2	1	2	1	2	
1	(10)	23.0 \pm 28.0	17.3 \pm 21.0	12.6 \pm 16.0	14.5 \pm 18.7	7.5 \pm 8.9	11.3 \pm 15.8	7.5 \pm 8.9	11.3 \pm 15.8	7.5 \pm 8.9	7.5 \pm 8.9
2	(10)	23.4 \pm 21.9	17.9 \pm 21.0	15.8 \pm 19.8	12.3 \pm 8.9	8.4 \pm 6.5	7.1 \pm 4.9	8.4 \pm 6.5	7.1 \pm 4.9	8.4 \pm 6.5	7.1 \pm 4.9
3	(8)	25.7 \pm 9.5	17.1 \pm 7.1	17.9 \pm 12.6	18.4 \pm 12.0	11.7 \pm 4.9	11.2 \pm 4.9	11.7 \pm 4.9	11.2 \pm 4.9	11.7 \pm 4.9	11.2 \pm 6.3
4	(11)	17.8 \pm 7.2	14.8 \pm 7.8	11.9 \pm 7.8	11.1 \pm 6.9	8.0 \pm 4.8	8.6 \pm 4.8	8.0 \pm 4.8	8.6 \pm 4.8	8.0 \pm 4.8	8.6 \pm 5.9
5	(10)	28.8 \pm 14.0	19.4 \pm 5.9	20.7 \pm 7.1	17.7 \pm 8.6	8.1 \pm 6.1	16.1 \pm 13.0	8.1 \pm 6.1	16.1 \pm 13.0	8.1 \pm 6.1	16.1 \pm 13.0

TABLE 1

Mean number of grains per 625 u^2 by segment 30 seconds after the application of morphine. n = number of separate tissues sampled; P.C. = posterior column; V.H. = ventral horn; I, II, V, VII = respective laminae.

GRAINS PER 625 μ^2 (mean \pm SD) 5 minutes

SEGMENT (n)	P.C.	I			II			V			VII			V.H.		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 (11)	17.0 \pm 15.2	7.8 \pm 6.6	8.3 \pm 6.3	5.0 \pm 5.4	3.5 \pm 3.6	4.9 \pm 4.9										
2 (10)	15.7 \pm 8.7	9.0 \pm 3.2	8.7 \pm 4.6	6.5 \pm 6.3	4.3 \pm 5.9	5.0 \pm 5.7										
3 (10)	28.2 \pm 18.0	18.4 \pm 10.0	14.3 \pm 9.3	8.7 \pm 9.1	6.1 \pm 6.0	6.6 \pm 5.3										
4 (10)	28.1 \pm 19.0	18.2 \pm 15.6	16.2 \pm 13.1	5.9 \pm 5.5	4.0 \pm 3.7	3.5 \pm 3.6										
5 (11)	28.9 \pm 22.0	13.5 \pm 11.4	12.1 \pm 11.5	5.6 \pm 5.5	3.7 \pm 4.5	4.9 \pm 7.5										

TABLE 2
Mean number of grains per 625 μ^2 by segment 5 minutes after the application of morphine.
See table 1 for legend.

GRAINS PER 625 μ^2		(mean \pm SD)		10 minutes			
SEGMENT	(n)	P.C.		I	II	V	VII
1	(10)	94.6 \pm 90.0	54.9 \pm 43.9	46.4 \pm 38.7	32.5 \pm 44.0	22.6 \pm 36.6	18.9 \pm 20.0
2	(10)	27.3 \pm 28.0	15.1 \pm 13.0	12.6 \pm 11.6	14.6 \pm 9.6	12.1 \pm 6.0	14.1 \pm 11.0
3	(10)	41.2 \pm 27.8	33.0 \pm 22.0	30.7 \pm 22.8	36.9 \pm 15.6	36.6 \pm 23.5	34.5 \pm 22.5
4	(10)	38.9 \pm 40.9	29.7 \pm 35.0	24.4 \pm 28.4	26.0 \pm 28.0	21.6 \pm 24.0	18.9 \pm 24.5
5	(8)	41.3 \pm 34.0	26.5 \pm 27.0	21.0 \pm 21.1	11.6 \pm 12.7	9.5 \pm 9.5	6.6 \pm 6.2

TABLE 3
 Mean number of grains per 625 μ^2 by segment 10 minutes after the application of morphine.
 See table 1 for legend.

GRAINS PER 625 u^2
(mean \pm SEM)

TIME	(n)	P.C.	I			II			V			VII			V.H.			
			17.2	\pm	2.0	15.6	\pm	1.9	14.6	\pm	1.7	8.6	\pm	0.9	10.8	\pm	1.5	
30 secs	(49)	23.5	\pm	2.5	17.2	\pm	2.0	15.6	\pm	1.9	14.6	\pm	1.7	8.6	\pm	0.9	10.8	\pm
5 mins	(52)	23.5	\pm	2.4	13.3	\pm	1.5	11.9	\pm	1.3	6.3	\pm	0.9*	4.3	\pm	0.7*	5.0	\pm
10 mins	(48)	49.0	\pm	7.8*	32.1	\pm	4.6*	27.3	\pm	4.0*	24.8	\pm	3.8*	20.9	\pm	3.5*	20.5	\pm

TABLE 4
Mean grains per 625 u^2 for each area based on pooled data from all segments taken together for each time interval. Legend as in table 1. * indicates statistical significance with $P < 0.01$ by the t-test for unpaired data.

REFERENCES

1. Atweh, S.F. and Kuhar, M.J.: Autoradiographic localization of opiate receptors in rat brain I. Spinal cord and lower medulla. *Brain Research* 124:53-67, 1977.
2. Basbaum, A.I. and Fields, H.L.: Endogenous pain control mechanisms: review and hypothesis. *Annals of Neurology* 4:451-462, 1978.
3. Besson, J.M., Wyon-Maillard, M.C., Benoist, J.M., Conseiller, C., and Hamann, K.F.: Effects of phenoperidine on lamina V cells in the cat dorsal horn. *J. Pharmacol. Exp. Ther.* 187:239-245, 1973.
4. Bromage, P.R.: The price of intraspinal narcotic analgesia: basic constraints. *Anesthesia and Analgesia* 60:461-463, 1981.
5. Burgen, A. in *Essentials of Molecular Pharmacology, Background for Drug Design*. ed. Korolkovas, A. [Wiley, N.Y.], 1970, p.123.
6. Calvillo, O., Henry, J.L., and Neuman, R.S.: Effects of morphine and naloxone on dorsal horn neurons in the cat. *Can. J. Physiol. Pharmacol.* 52:1207-1211, 1974.
7. Clark, S.L., Edeson, R.O., and Ryall, R.W.: The relative significance of spinal and supraspinal actions in the antinociceptive effect of morphine in the dorsal horn: an evaluation of the microinjection technique. *Br. J. Pharmac.* 79:807-818, 1983.
8. Clark, S.L. and Ryall, R.W.: The antinociceptive action of etorphine in the dorsal horn is due to a direct spinal action and not to activation of descending inhibition. *Br. J. Pharmac.* 78:307-319, 1983.
9. Cousins, M.J. and Mather, L.E.: Intrathecal and epidural opioids. *Anesthesiology* 61:276-310, 1984.
10. Cox, B.M.: Endogenous opioid peptides: a guide to structures and terminology. *Life Sci.* 31:1645-1658, 1982.
11. Cox, B.M., Goldstein, A. and Li, C.H.: Opioid activity of a peptide, B-lipotropin-(61-91), derived from B-lipotropin. *Proc. Natl. Acad. Sci. USA* 73:1821-1823, 1976.

12. Cox, B.M., Opheim, K.E., Teschemacher, H. and Goldstein, A.: A peptide-like substance from pituitary that acts like morphine. 2. Purification and properties. *Life Sci.* 16:1777-1782, 1975.
13. Creese, I. and Snyder, S.H.: Receptor binding and pharmacological activity of opiates in the guinea-pig intestine. *J. Pharmacol. Exp. Ther.* 194:205-219, 1975.
14. Dohi, S., Toyooka, H. and Kitahata, L.M.: Effects of morphine sulfate on dorsalhorn neuronal responses to graded noxious thermal stimulation in the decerebrate cat. *Anesthesiology* 51:408-413, 1979.
15. Duggan, A.W., Hall, J.G. and Headley, P.M.: Suppression of transmission of nociceptive impulses by morphine: selective effects of morphine administered in the region of the substantia gelatinosa. *Br. J. Pharmac.* 61:65-76, 1977.
16. Duggan, A.W. and North, R.A.: Electrophysiology of Opioids. *Pharmac. Reviews* 35:219-281, 1983.
17. Editorial. *Anaesthesia* 37:527-529, 1982.
18. Fields, H.L. and Basbaum, A.I.: Brainstem control of spinal pain-transmission neurons. *Ann. Rev. Physiol.* 40:217-248, 1978.
19. Fields, H.L., Emson, P.C., Leigh, B.K., Gilbert, R.F.T. and Iversen, L.L.: Multiple opiate receptor sites on primary afferent fibres. *Nature* 284:351-353, 1980.
20. Gahan, P.B.: *Autoradiography for Biologists*. [Academic Press, New York], 1972.
21. Goldsmith, M.: *The Trail of Opium*. [Hale, London], 1939.
22. Goldstein, A., Lowney, L.I. and Pal, B.K.: Stereospecific and nonspecific interaction of the morphine co-agonist levorphanol in subcellular fractions of mouse brain. *Proc. Natl. Acad. Sci. USA* 68:1742-1747, 1971.
23. Goldstein, A., Tachibana, S., Lowney, L.I., Hunkapiller, M. and Hood, L.: Dynorphin-(1-13), and extraordinarily potent opioid peptide. *Proc. Natl. Acad. Sci. USA* 76:6666-6670, 1979.

24. Goodman, R.R., Snyder, S.H., Kuhar, M.J. and Young, W.S.III: Differentiation of delta and mu opiate receptor localizations by light microscopic autoradiography. *Proc. Natl. Acad. Sci. USA* 77:6239-6243, 1980.
25. Goodman-Gilman, A., Goodman, L.S. and Gilman, A.: *The Pharmacological Basis of Therapeutics.* [Macmillan, N.Y.], 1980.
26. Gustafsson, L.L., Post, C., Edvardsen, B. and Ramsay, C.H.: Distribution of morphine and meperidine after intrathecal administration in rat and mouse. *Anesthesiology* 63:483-489, 1985.
27. Hanaoka, K., Ohtani, M., Toyooka, H., Dohi, S., Ghazi-Saidi, K., Taub, A. and Kitahata, L.M.: The relative contribution of direct and supraspinal descending effects upon spinal mechanisms of morphine analgesia. *J. Pharmacol. Exp. Ther.* 207:476-484, 1978.
28. Heavner, J.E.: Jamming spinal sensory input: effects of anesthetic and analgesic drugs in the spinal cord dorsal horn. *Pain* 1:239-255, 1975.
29. Herkenham, M. and Pert, C.B.: Light microscopic localization of brain opiate receptors: a general autoradiographic method which preserves tissue quality. *J. Neuroscience* 2:1129-1149, 1982.
30. Herz, A. and Teschemacher, H.J.: Activities and site of antinociceptive action of morphine-like analgesics and kinetics of distribution following intravenous, intrathecal, and intraventricular application. *Adv. Drug Res.* 6:79-119, 1971.
31. Hiller, J.M., Pearson, J. and Simon, E.J.: Distribution of stereospecific binding of the potent narcotic analgesic etorphine in the human brain: predominance in the limbic system. *Res. Comm. Chem. Path. Pharm.* 6:1052-1061, 1973.
32. Homma, E., Collins, J.G., Kitahata, L.M., Matsumoto, M. and Kawahara, M.: Suppression of noxiously evoked WDR dorsal horn neuronal activity by spinally administered morphine. *Anesthesiology* 58:232-236, 1983.
33. Hosobuchi, Y., Rossier, J., Bloom, F.E. and Guillemin, R.: Stimulation of human periaqueductal gray for pain relief increases immunoreactive B-endorphin in ventricular fluid. *Science* 203:279-281, 1979.

34. Hughes, J.: Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. *Brain Research* 88:295-308, 1975.
35. Hughes, J., Smith, T.W., Kosterlitz, H.W., Fothergill, L.A., Morgan, B.A. and Morris, H.R.: Identification of two related pentapeptides from the brain with potent opiate agonist activity. *Nature* 258:577-579, 1975.
36. Kerr, F.W.L.: Neuroanatomical substrates of nociception in the spinal cord. *Pain* 1:325-356, 1975.
37. Kitahata, L.M. and Collins, J.G.: Spinal action of narcotic analgesics. *Anesthesiology* 54:153-163, 1981.
38. Kitahata, L.M., Kosaka, Y., Taub, A., Bonikos, K. and Hoffert, M.: Lamina-specific suppression of dorsal-horn unit activity by morphine sulfate. *Anesthesiology* 41:39-48, 1974.
39. Koll, W., Haase, J., Block, G. and Muhlberg, B.: The predilective action of small doses of morphine on nociceptive spinal reflexes of low spinal cats. *Intl. J. Neuropharmacol.* 2:57-65, 1963.
40. Kuhar, M.J., Pert, C.B. and Snyder, S.H.: Regional distribution of opiate receptor binding in monkey and human brain. *Nature* 245:447-450, 1973.
41. Kumeta, Y., Senami, M., Aoki, M., Kitahata, L.M. and Collins, J.G.: Direct opioid application on peripheral nerves does not alter activity of single C polymodal nociceptive fibers. *Anesthesiology* 63:A233, 1985.
42. LaMotte, C., Pert, C.B. and Snyder, S.H.: Opiate receptor binding in primate spinal cord: distribution and changes after dorsal root section. *Brain Research* 112:407-412, 1976.
43. Le Bars, D., Menetrey, D., Conseiller, C. and Besson, J.M.: Depressive effects of morphine upon lamina V cells activities in the dorsal horn of the spinal cat. *Brain Research* 98:261-277, 1975.
44. Li, C.H., Barnafi, L., Chretten, M. and Chung, D.: Isolation and amino-acid sequence of B-LPH from sheep pituitary glands. *Nature* 208:1093-1094, 1965.
45. Li, C.H. and Chung, D.: Isolation and structure of an untriacontapeptide with opiate activity from camel pituitary glands. *Proc. Natl. Acad. Sci. USA* 73:1145-1148, 1976.

46. Loh, H.H., Cho, T.M., Wu, Y.C. and Way, E.L.: Stereo-specific binding of narcotics to brain cerebrosides. *Life Sci.* 14:2231-2245, 1974.
47. Lord, J.A., Waterfield, A.A., Hughes, J and Kosterlitz, H.W.: Endogenous opioid peptides: multiple agonists and receptors. *Nature* 267:495-499, 1977.
48. Martin, W.R., Eades, C.G., Thompson, J.A., Huppler, R.E. and Gilbert, P.E.: The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. *J. Pharmacol. Exp. Ther.* 197:517-532, 1976.
49. Matsuki, A.: Nothing new under the sun--a Japanese pioneer in the clinical use of intrathecal morphine. *Anesthesiology* 58:289-290, 1983.
50. Mayer, D.J. and Price, D.D.: Central nervous system mechanisms of analgesia. *Pain* 2:379-404, 1976.
51. Pasternak, G.W., Goodman, R. and Snyder, S.H.: An endogenous morphine-like factor in mammalian brain. *Life Sci.* 16:1765-1769, 1975.
52. Pert, C.B., Kuhar, M.J. and Snyder, S.H.: Autoradiographic localization of the opiate receptor in rat brain. *Life Sci.* 16:1849-1854, 1975.
53. Pert, C.B., Kuhar, M.J. and Snyder, S.H.: Opiate receptor: autoradiographic localization in rat brain. *Proc. Natl. Acad. Sci. USA* 73:3729-3733, 1976.
54. Pert, C.B. and Snyder, S.H.: Opiate receptor: demonstration in nervous tissue. *Science* 179:1011-1014, 1973.
55. Rogers, A.W.: Techniques of Autoradiography. [Elsevier, Amsterdam], 1979.
56. Roth, L.J. and Stumpf, W.E.: Autoradiography of Diffusible Substances. [Academic Press, N.Y.], 1969.
57. Satoh, M. and Takagi, H.: Enhancement by morphine of the central descending inhibitory influence on spinal sensory transmission. *Eur. J. Pharmacol.* 14:60-65, 1971.
58. Schmauss, C. and Yaksh, T.L.: In vivo studies on spinal opiate receptor systems mediating antinociception. II. Pharmacological profiles suggesting a differential association of mu, delta, and kappa receptors with visceral chemical and cutaneous thermal stimuli in the rat. *J. Pharmacol. Exp. Ther.* 228:1-12, 1984.

59. Scott, J.M.: *The White Poppy*. [Heinemann, London], 1969.
60. Simon, E.J., Hiller, J.M. and Edelman, I.: Stereospecific binding of the potent narcotic analgesic [³H]etorphine to rat brain homogenate. *Proc. Natl. Acad. Sci. USA* 70:1947-1949, 1973.
61. Snyder, S.H.: Opiate receptors and internal opiates. *Scientific American* 236:44-56, 1977.
62. Snyder, S.H., Pasternak, G.W. and Pert, C.B.: Opiate receptor mechanisms. *Handbook of Psychopharmac.* 5:329-360, 1975.
63. Snyder, S.H. and Simantov, R.: The opiate receptor and opioid peptides. *J. Neurochem.* 28:13-20, 1977.
64. Suzukiwa, M., Matsumoto, M., Collins, J.G., Kitahata, L.M. and Yuge, O.: Dose-response suppression of noxiously evoked activity of WDR neurons by spinally administered Fentanyl. *Anesthesiology* 58:510-513, 1983.
65. Terenius, L.: Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex. *Acta Pharmac. Toxic.* 32:317-320, 1973.
66. Terenius, L. and Wahlstrom, A.: Inhibitor(s) of narcotic receptor binding in brain extracts and cerebrospinal fluid. *Acta Pharmac. Toxic.* 35(suppl. 1):55, 1974.
67. Teschemacher, H., Opheim, K.E., Cox, B.M. and Goldstein, A.: A peptide-like substance from pituitary that acts like morphine. 1. Isolation. *Life Sci.* 16:1771-1776, 1975.
68. Toyooka, H., Hanaoka, K., Ohtani, M., Yamashita, M., Taub, A. and Kitahata, L.M.: Suppressive effect of morphine on single-unit activity of cells in Rexed lamina VII. *Anesthesiology* 47:513-517, 1977.
69. Toyooka, H., Kitahata, L.M., Dohi, S., Ohtani, M., Hanaoka, K. and Taub, A.: Effects of morphine on the Rexed lamina VII spinal neuronal response to graded noxious radiant heat stimulation. *Exp. Neurology* 62:146-158, 1978.
70. Traynor, J.R., Kelly, P.D. and Rance, M.J.: Multiple opiate binding sites in rat spinal cord. *Life Sci.* 31:1377-1380, 1982.

71. Tung, A.S. and Yaksh, T.L.: In vivo evidence for multiple opiate receptors mediating analgesia in the rat spinal cord. *Brain Research* 247:75-83, 1982.
72. Wang, J.K., Nauss, L.A. and Thomas, J.E.: Pain relief by intrathecally applied morphine in man. *Anesthesiology* 50:149-151, 1979.
73. Wikler, A.: Studies on the action of morphine on the central nervous system of cat. *J. Pharmacol. Exp. Ther.* 80:176-187, 1944.
74. Wuster, M., Schulz, R. and Herz, A.: Specificity of opioids towards the u-, d-, and e-opiate receptors. *Neurosci. Letters* 15:193-198, 1978.
75. Yaksh, T.L.: Analgetic Actions of intrathecal opiates in cat and primate. *Brain Research* 153:205-210, 1978.
76. Yaksh, T.L.: Spinal opiate analgesia: characteristics and principles of action. *Pain* 11:293-346, 1981.
77. Yaksh, T.L. and Noueihed, R.: The physiology and pharmacology of spinal opiates. *Ann. Rev. Pharmacol. Toxic.* 25:433-462, 1985.
78. Yaksh, T.L. and Rudy, T.A.: Analgesia mediated by a direct spinal action of narcotics. *Science* 192:1357-1358, 1976.
79. Yaksh, T.L. and Rudy, T.A.: Studies on the direct spinal action of narcotics in the production of analgesia in the rat. *J. Pharmacol. Exp. Ther.* 202:411-428, 1977.
80. Yamamura, H.I. and Enna, S.J. (eds.): *Neurotransmitter receptor binding*. 2nd ed. [Raven Press, N.Y.], 1985.
81. Yuge, O., Matsumoto, M., Kitahata, L.M., Collins, J.G. and Senami, M.: Direct opioid application to peripheral nerves does not alter compound action potential. *Anesthesia & Analgesia* 64:667-671, 1985.

d
he

t

2

YALE MEDICAL LIBRARY

11

10

YALE MEDICAL LIBRARY



3 9002 01005 0905

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been
used by the following persons, whose signatures attest their acceptance of the
above restrictions.

D. K. Saad (M.D.)

Consultant of Anesthesia, Hamad General Hospital (S.I.C.U.)

NAME AND ADDRESS

P.O.B. 3450
DOHA - QATAR.

DATE

27/8/88

